Amendments to the Specification

At the indicated page and line numbers, please replace the existing sections or paragraphs, as the case may be, with the ones set forth below.

(Page 1, line 1 and Title)

~~POLYKETIDES AND THEIR SYNTHESIS~~

DNA Encoding MonAIV of the Monensin Polyketide Synthase Gene Cluster

(Page 21, lines 12-20)

Brief Description of the Drawings

Some embodiments of the invention will now be described by way of example with reference to the accompanying drawings in which:

Fig 1 shows the structure of monensins A and B;

Fig 2 illustrates proposed biosynthetic pathways;

Fig 3 illustrates the proposed organization of the monensin polyketide synthase (PKS) enzyme complex; and

Fig 4 illustrates the proposed organization of the monensin biosynthetic gene cluster.

(Page 39, line 22 through page 40, line 13)

The DNA of each cosmid was separately subjected to partial digestion with *Sau*3A and fragments of approximately 1.5-2.0 kbp were separated by agarose gel electrophoresis. The fragments were then ligated into the plasmid vector pUC18 ~~(Messing, 1982)~~, previously digested with *Bam*HI and treated with shrimp alkaline phosphatase. The library was transformed into *E. coli* strain XL1-Blue MR and plated on 2xTY agar medium containing ampicillin (100 µg/ml) to select for plasmid-containing cells. Plasmid DNA was purified from individual colonies and sequenced using the Sanger dye-terminator procedure on an ABI 377 automated sequencer (Sanger, F.

Science (1981) 214:1205-1210). The sequence data obtained from single random subclones of a cosmid was assembled into a single continuous sequence and edited using GAP4.1 program of the STADEN gene analysis package (Staden, R. Molecular Biotechnology (1996) 5:233-241).

(Page 44, line 13 through page 45, line 6)

The ermE* promoter derived from the *ermE* resistance methyltransferase gene of *S. erythraea* (Bibb et al. Gene (1985) 38:215-226) was amplified by PCR as a *Spe*I-*Xba*I fragment using the following oligonucleotides 5'-CCACTAGTATGCATGCGAGTGTCCGTTCGAGT-3' (SEQ ID NO: 39) and 5'-TTGTATACACCTAGGATGGTTGGCCGTGC-3' (SEQ ID NO: 40) with pRH3 (Dhillon et al. Molecular Microbiology (1989) 3:1405-1414 as a template and cloned into *Sma*I-digested, phosphatase-treated pUC18, to produce plasmid pIB135. The integrative plasmid pSET152 (Bierman, M. et al. (1992) Gene 116:43-49)) was digested with *Xba*I and the backbone was dephosphorylated and ligated to the *Spe*I-*Xba*I fragment of pIB135 containing the ermE* promoter. The ligation mixture was used to transform *E. coli* DH10B and the orientation of the insert in the plasmids from individual clones was checked by using restriction analysis. A plasmid with the ermE* promoter oriented so that the *Nde*I and *Xba*I sites are adjacent to the apramycin resistance gene was selected and named pIB139.

(Page 45, lines 7-20)

The *monR* gene from the monensin biosynthetic gene cluster was amplified and *Nde*I and *Xba*I restriction sites introduced at 5' and 3' ends respectively, by PCR using as primers the following oligonucleotides: 5'-AGA TAC CAT ATG CTG GGC CCG CTC CGC AT-3' (SEQ ID NO: 41) and 5'-AAT GCT CTA GAC TGT CAG CGA CCG GAC AGG GCC AA-3' (SEQ ID NO: 42) and cosmid MO.CN11 as template. The PCR product was ligated into *Sma*I-treated and

phosphatase-treated plasmid pUC18 and the ligation mixture was used to transform *E. coli* DH10B cells. Transformant colonies were analysed for the presence of plasmid and the identity of the plasmid inserts was verified by sequencing. A plasmid whose insert contained the *monR* gene flanked by *Nde*I and *Xba*I restriction sites was selected and designated pCJW57.

(Page 50, line 25 through page 52, line 2)

A region lying immediately 5' of the DNA encoding the acyltransferase (AT12) domain of module 12 of the monensin polyketide synthase in the monensin biosynthetic gene cluster was amplified with the following primers : 5'-GGTGGCCACGGAAACACCAACACCGGACCCGCGCC-3' (SEQ ID NO: 43), and 5'-CTCTCGGAGGCCCGGCGCAACGGCCACAA-3' (SEQ ID NO: 44), 3' using cosmid MO-CN11 as a template. The PCR product was ligated into *Sma*I digested and phosphatase-treated plasmid pUC18 and the ligation mixture was used to transform *E. coli* DH10B cells. Transformant colonies were analysed for the presence of plasmid and the identity of the plasmid inserts was verified by sequencing. A plasmid whose insert contained a fragment upstream of the AT12-encoding sequence from about 82.3kb to 83.2kb of the *mon* cluster was designated pMO81. Similarly a region lying immediately 3' of the DNA encoding the acyltransferase (AT12) domain of module 12 of the monensin polyketide synthase in the monensin biosynthetic gene cluster was amplified with the following primers : 5'-GGCCTAGGGCTGCCTCGGGTGGTGGATCTGCCGA-3' (SEQ ID NO: 45) and 5'-TGGTCGGGCGCGGTGCGTGCGATACGT-3' (SEQ ID NO: 46), using cosmid MO-CN11 as a template. The PCR product was ligated into *Sma*I-treated and dephosphorylated pUC18 AND the ligation mixture was used to transform DH10B *E. coli* cells. Transformant colonies were analysed for the presence of plasmid and the identity of the plasmid inserts was verified by sequencing. A plasmid whose insert contained a fragment downstream of the AT12-encoding sequence, from 80.5kb to 81.4kb of the *mon*

cluster, was designated pMO82.

(Page 52, lines 3-15)

The DNA encoding AT of module 5 was amplified and *Msc*I and *Avr*II restriction enzyme recognition sites were introduced at the ends by PCR using the following primers : 5'-CCTGGCCAGGGCGGCCAGTGGGTGGGCATG-3' (SEQ ID NO: 47) and 5'-GGCCTAGGGGTCGGCCGGGAACCAGCGCCGCCAGT-3' (SEQ ID NO: 48) and the cosmid MO-CN33 as a template. The PCR product was ligated into *Sma*I-treated and dephosphorylated pUC18 and the ligation mixture was used to transform DH10B *E. coli* cells. Transformant colonies were analysed for the presence of plasmid and the identity of the plasmid inserts was verified by sequencing. A plasmid whose insert DNA, with sequence from about 44.2kb to 45.2kb of the mon cluster, encoded the AT5 domain was designated pMO83.

(Page 56, lines 10-14)

A 2769bp DNA segment of the monensin cluster of *S. cinnamonensis* extending from nucleotide 42438 to 45207 was amplified by PCR using the following oligonucleotide primers. 5'-GTGACGTCATATGTCGAGTGCTGAAGAGTCG-3' (SEQ ID NO: 49) and 5'-GGGGTCGCCTAGGAACCAGCGCCGCCAGTCGA-3' (SEQ ID NO: 50).

(Page 56, lines 21-25)

A second PCR reaction was used with the same template to amplify DNA from nucleotide 43098 to 45207. The primers used were 5'-CGGCCTCGAGGGCCCGTCGGTCAGTGTCGACACGGCGCAGTCCTCCTCGC-3' (SEQ ID NO: 51) and 5'-GGGGTCGCCTAGGAACCAGCGCCGCCAGTCGA-3' (SEQ ID NO: 52).

(Page 68, line 1)

TABLE I

(start and end nucleotides refer to the monensin biosynthetic gene cluster provided as SEQ ID NOs: 1-4)

Please delete page 70, line 1 through page 99, line 24 and replace with the following:

TABLE II

(Table II is an abbreviated version of Table II of pages 70-99 of PCT/GB00/02072, which is incorporated herein by reference.)

GdhA, glutamate dehydrogenase (partial coding sequence)
Length: 346 amino acids, SEQ ID NO: 5

DapA, dihydrodopicolinate synthase
Length: 307 amino acids, SEQ ID NO: 6

ORF3, putative transcriptional activator protein
Length: 314 amino acids, SEQ ID NO: 7

ORF4, hypothetical protein
Length: 139 amino acids, SEQ ID NO: 8

ORF5, hypothetical protein
Length: 208 amino acids, SEQ ID NO: 9

ORF6, hypothetical protein
Length: 63 amino acids, SEQ ID NO: 10

ORF7, hypothetical protein
Length: 185 amino acids, SEQ ID NO: 11

AcpX, acyl carrier protein (ACP)
Length: 106 amino acids, SEQ ID NO: 12

KsX, ketoacyl-ACP synthase
Length: 829 amino acids, SEQ ID NO: 13

MonCII, probable epoxyhydrolase/cyclase
Length: 300 amino acids, SEQ ID NO: 14

MonE, S-adeonosylmethionine-dependent methyltransferase
Length: 277 amino acids, SEQ ID NO: 15

MonT, putative monensin resistance gene (ABC-transporter)
Length: 512 amino acids, SEQ ID NO: 16

MonRII, probable repressor protein

Length: 192 amino acids, SEQ ID NO: 17

MonAIX, thioesterase

Length: 269 amino acids, SEQ ID NO: 18

MonAI, polyketide synthase multi-enzyme MONS1, housing loading module and extension module 1

Length: 3026 amino acids, SEQ ID NO: 19

MonAII, polyketide synthase multi-enzyme MONS2, housing extension module 2

Length: 2239 amino acids, SEQ ID NO: 20

MonAIII, polyketide synthase multi-enzyme MONS3, housing extension modules 3 and 4

Length: 4133 amino acids, SEQ ID NO: 21

MonAIV, polyketide synthase multi-enzyme MONS4, housing extension modules 5 and 6

Length: 4039 amino acids, SEQ ID NO: 22

MonAV, polyketide synthase multi-enzyme MONS5, housing extension modules 7 and 8

Length: 4107 amino acids, SEQ ID NO: 23

MonAVI, polyketide synthase multi-enzyme MONS6, housing extension module 9

Length: 1701 amino acids, SEQ ID NO: 24

MonH, probable regulatory protein

Length: 981 amino acids, SEQ ID NO: 25

MonCI, flavin-dependent epoxidase

Length: 496 amino acids, SEQ ID NO: 26

MonBII, carbon-carbon double bond isomerase

Length: 141 amino acids, SEQ ID NO: 27

MonBI, carbon-carbon double bond isomerase

Length: 144 amino acids, SEQ ID NO: 28

MonAVIII, polyketide synthase multi-enzyme MONS8, housing extension modules 11 and 12

Length: 3754 amino acids, SEQ ID NO: 29

MonAVII, polyketide synthase multi-enzyme MONS7, housing extension module 10

Length: 1642 amino acids, SEQ ID NO: 30

MonD, cytochrome P450 hydroxylase

Length: 431 amino acids, SEQ ID NO: 31

MonRI, probable activator protein

Length: 268 amino acids, SEQ ID NO: 32

MonAX, thioesterase

Length: 278 amino acids, SEQ ID NO: 33

ORF29, a homologue of CapK involved in cell wall biosynthesis

Length: 428 amino acids, SEQ ID NO: 34

LipB, lipase B

Length: 338 amino acids, SEQ ID NO: 35

ORF31, putative ion pump

Length: 309 amino acids, SEQ ID NO: 36

ORF32, hypothetical membrane protein

Length: 364 amino acids, SEQ ID NO: 37

AmtA, glycine amidinotransferase (partial coding sequence)

Length: 131 amino acids, SEQ ID NO: 38